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Closely Related Tree Species Differentially Influence the Transfer of Carbon and Nitrogen from Leaf Litter Up the Aquatic Food Web

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Abstract

Decomposing leaf litter in streams provides habitat and nutrition for aquatic insects. Despite large differences in the nutritional qualities of litter among different plant species, their effects on aquatic insects are often difficult to detect. We evaluated how leaf litter of two dominant riparian species (*Populus fremontii* and *P. angustifolia*) influenced carbon and nitrogen assimilation by aquatic insect communities, quantifying assimilation rates using stable isotope tracers (^{13}C, ^{15}N). We tested the hypothesis that element fluxes from litter of different plant species better define aquatic insect community structure than insect relative abundances, which often fail. We found that (1) functional communities (defined by fluxes of carbon and nitrogen from leaf litter to insects) were different between leaf litter species, whereas more traditional insect communities (defined by relativized taxa abundances) were not different between leaf litter species, (2) insects assimilated N, but not C, at a higher rate from *P. angustifolia* litter compared to *P. fremontii*, even though *P. angustifolia* decomposes more slowly, and (3) the C:N ratio of material assimilated by aquatic insects was lower for *P. angustifolia* compared to *P. fremontii*, indicating higher nutritional quality, despite similar initial litter C:N ratios. These findings provide new evidence for the effects of terrestrial plant species on aquatic ecosystems via their direct influence on the transfer of elements up the food web. We demonstrate how isotopically labeled leaf litter can be used to assess the functioning of insect communities, uncovering patterns undetected by traditional approaches and improving our understanding of the association between food web structure and element cycling.

Key words: stable isotope tracers; functional food webs; trophic structure; nutrient cycling; decomposition; cottonwood; aquatic insect community.

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Author contributions ZGC, BAH, JCM, GWK, and SCH contributed to designing the study. TGW established and maintained the common garden. ZGC and KJA designed and constructed labeling chambers, and ZGC, KJA, and JMM grew and harvested labeled leaves. BAH, GWK, and SCH assisted ZGC with developing labeling techniques. ZGC and BAH developed flux equations and ZGC, BAH, and JMM performed related analyses. ZGC wrote the first draft of the manuscript, and JCM, BAH, TGW, GWK, and SCH contributed substantially to revisions.

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Introduction

Food webs depict quantitative relationships of energy flow among organisms in biological communities and ecosystems (Lindeman 1942). Since Watt's (1947) seminal paper, ecologists have attempted to disentangle process from pattern in food webs, but quantifying these relationships remains challenging. Often, the abundances of community members can be measured, but quantifying their activities is more difficult. Invertebrate assemblages occurring on decomposing leaf litter are frequently measured in stream ecosystems, where leaf litter is a major source of energy, in an effort to understand which organisms contribute to decomposition or which organisms benefit most from the decomposing litter substrate (LeRoy and Marks 2006; Wallace and others 1982; Cummins and others 1989). The relationships between invertebrates and leaf litter quality, however, are often unclear (LeRoy and others 2007; Dudgeon and Gao 2011; Li and others 2009). One challenge is that insects inhabit litter because it can be an important source of nutrition and shelter, but also because of random processes such as invertebrate drift. Thus, the mere presence of an organism on decomposing litter may not reflect the insect's reliance on that litter for nutrients. For this reason, the insect community structure assessed by abundances of taxa may not accurately reflect functional food web interactions.

Litter traits that correlate with decomposition rate, such as N and P concentrations, lignin, tannins, leaf toughness, C:N, and lignin:N (Fogel and Cromack 1977; Melillo and others 1982), reflect the nutrient content and energy availability of the litter substrate, and thus may indicate its nutritional value to decomposer invertebrates. In addition to predicting the decomposition rate of the litter substrate, these indices may also provide information about how quickly nutrients bound in leaf litter enter the stream food web.

We used double-labeled (^{13}C and ^{15}N) litter of Fremont (*Populus fremontii*) and narrowleaf (*P. angustifolia*) cottonwood to measure rates of C and N assimilation into aquatic insects occurring on these litter types in a headwater stream. We also determined the structure of those communities using proportional abundances of insect taxa. Leaf litter from our study trees has different traits: *P. fremontii* has lower lignin and tannin concentrations and decomposes more quickly than *P. angustifolia* litter (Table 1; LeRoy and others 2006, 2007; Schweitzer and others 2004; Holeski and others 2012). We postulated that insect communities defined by C and N assimilation rates would be more sensitive to differences in the litter types than would communities defined by insect abundances, because C and N assimilation more directly measures the reliance of the invertebrates on the litter substrates for nutrition. We also postulated that insects would assimilate C and N more rapidly from *P. fremontii* compared to *P. angustifolia* litter because *P. fremontii* litter decomposes more rapidly, presumably making C and N more rapidly accessible to insects, and because a relatively large proportion of C and N in *P. angustifolia* litter is bound up in recalcitrant compounds.

Methods

Cottonwood Study System

We used two species of cottonwood, a common riparian tree throughout the western U.S., providing as much as 93% of the litter inputs (Driebe and Whitham 2000), making them potential drivers of aquatic processes. Leaf litter and cuttings were harvested from trees grown in a common garden at the Ogden Nature Center, Utah, which allowed us to isolate species effects from environmental effects. Differences in litter phytochemistry and decomposition are well documented and predictable: *P. fremontii* litter contains lower lignin and tannins and consistently decomposes faster than *P. angustifolia* litter (Schweitzer and others 2004; LeRoy and others 2006, 2007; Holeski and others 2012; Table 1).

Greenhouse Set-Up and Labeling Schemes

In January of 2007 we rooted 10-cm-long cuttings of *P. fremontii* and *P. angustifolia* (n = 128 trees total) from the common garden and later transferred them to five-gallon pots at the Northern Arizona University Research Greenhouse. Plants were assigned random positions on greenhouse benches and shuffled two times per week to minimize environmental variability in microclimate. The greenhouse air temperature was approximately 24°C during the day and 18°C during the night. In the fall, greenhouse temperatures were dropped to ~10°C and ~4.4°C for daytime and nighttime temperatures, respectively, to promote leaf senescence. Plants were fertilized with 60 ppm Peters Professional Water Soluble 20-20-20 (NPK) fertilizer with micronutrients (The Scotts Company, Inc., Marysville, Ohio, USA). Fertilizer was applied using a Dosmatic Advantage A20–2.5% mixer–proportioner attached to a garden hose, with the

Table 1. Initial Chemistry and Aquatic Decomposition Values (k, d^{-1}) for Senescent *Populus fremontii* and *P. angustifolia* Cottonwood Leaf Litter

Variable	*P. fremontii*	*P. angustifolia*	Citation
CT (%)	$0.058^a \pm 0.02$	$9.261^b \pm 1.80$	LeRoy and others (2006)
	$0.162^a \pm 0.04$	$9.639^b \pm 1.674$	LeRoy and others (2007)
Lignin (%)	$5.20^a \pm 0.29$	$20.33^b \pm 0.91$	LeRoy and others 2007
%C	$40.6^a \pm 0.50$	$45.0^b \pm 0.31$	This study
%N	$0.433^a \pm 0.01$	$0.453^a \pm 0.01$	LeRoy and others (2006)
	$0.465^a \pm 0.03$	$0.511^a \pm 0.02$	LeRoy and others (2007)
	$2.17^a \pm 0.16$	$2.53^a \pm 0.18$	This study
%P	$0.043^a \pm 0.01$	$0.074^a \pm 0.01$	LeRoy and others (2006)
	$0.061^a \pm 0.02$	$0.144^a \pm 0.04$	LeRoy and others 2007
C:N ratio	$99.11^a \pm 2.8$	$103.80^a \pm 2.2$	LeRoy and others (2006)
	$93.96^a \pm 8.7$	$88.77^a \pm 4.6$	LeRoy and others (2007)
	$22.27^a \pm 1.6$	$24.30^a \pm 2.4$	This study
k (d^{-1})	$0.0162^a \pm 0.001$	$0.0117^b \pm 0.001$	LeRoy and others (2006)
	$0.0104^a \pm 0.0003$	$0.00878^b \pm 0.0004$	LeRoy and others (2007)
	$0.0581^a \pm 0.005$	$0.0403^b \pm 0.003$	This study

Differing letters designate statistical differences within a row using Tukey's HSD, Hommel's correction (LeRoy and others 2006, 2007), or Student's t tests (this study). ANCOVA with a Leaf Species × Time interaction tested differences in slopes for decomposition (k (d^{-1})).

bulk aqueous solution kept at pH 5.5–6.2 to optimize nutrient uptake. Soils were watered every other day to saturation. The fertilization provided nitrogen in excess of supplies likely found under field conditions, resulting in tissue nitrogen concentrations on the high end (*P. fremontii*: 2.26 ± 0.22%; *P. angustifolia*: 2.34 ± 0.19%) of values typically observed for cottonwoods (LeRoy and others 2006, 2007; Schweitzer and others 2005a, b) and related species (Tibbets and Molles 2005). Nitrogen concentrations of our litter were within the range of values found for conditioned litter (1.24–3.01%N), unconditioned fresh leaves (2.24–3.68%N), or conditioned fresh leaves (2.17–3.07%N) used in other studies that measured growth or assimilation in the laboratory (Friberg and Jacobsen 1994, 1999; Iversen 1979; Jacobsen and Sand-Jensen 1994; Table 2).

Plants were labeled from late May to late October of 2007, until leaves began senescing. Plants were labeled with C by placing them in 1.22 × 1.52 × 2.44 m airtight, steel-framed, Plexiglas chambers pulsed with 0.22 l m^{-3} 99 at.% ^{13}C–CO_2 twice a week for 4 h. The greenhouse was climate controlled during labeling to keep the internal chamber environment at approximately 27°C and about 90% relative humidity. Air temperature and relative humidity were monitored inside and outside labeling chambers during ^{13}C–CO_2 pulsing events. Additionally, CO_2 concentrations in the chambers were monitored periodically throughout the study. Plants were labeled with N by dripping approximately 0.003 g of 99 at.% ^{15}N ammonium sulfate ($(NH_4)_2SO_4$) in aqueous solution into pots twice a week after watering. Leaves were harvested as they senesced from October to December and pooled by species. Isotope labeling increased $\delta^{13}C$ by around 350‰ and $\delta^{15}N$ by around 6,200‰ for both *P. fremontii* and *P. angustifolia*. The strength of the isotope label did not differ between species, whether considering bulk litter ($P > 0.55$) or litter after all soluble compounds had been removed by boiling for an hour ($P > 0.45$). Thus, labeling provided strong isotope signals to compare how C and N flow from decomposing litter to macroinvertebrates differs between these tree species in our experiment.

Field Experiment

The field experiment was conducted from January to March of 2008. We chose this time period because it was after litter drop, when shredder diversity and density were relatively high for upper Oak Creek (5200 ft a.s.l.), AZ (35°02′N, 111°43′W). Oak Creek, a second order, perennial stream in the upper Verde River drainage (14,100 km^2), flows off the southwestern edge of the Colorado Plateau in north-central Arizona, USA. Riparian vegetation includes Fremont cottonwood (*P. fremontii* S. Wats.), narrowleaf cottonwood (*P. angustifolia* James), Arizona alder (*Alnus oblongifolia* Torr.), box elder (*Acer negundo* L.), Gambel oak (*Quercus gambelii* Nutt.), Arizona sycamore (*Platanus wrightii* S. Wats), velvet ash (*Fraxinus velutina* Torr.), coyote

Table 2. Assimilation Rates per Individual (ARI) (mg Mass Assimilated insect^{-1} d^{-1}) and Relative Assimilation Rates (RAR) (mg Mass Assimilated mg insect^{-1} d^{-1}) for Shredder Taxa Fed Food Sources (CLL = conditioned leaf litter, ULL = unconditioned leaf litter, UGL = unconditioned green leaves, UGM = unconditioned green macrophyte) with Varying Condition Times (CT (d)), Varying Nitrogen Content LNC (%N), and at Varying Water Temperatures (WT(°C))

Citation	Species	Instar	Size class (mg)	Food source	LT	LNC (%N)	CT (d)	WT (°C)	ARI	RAR
Golladay and others (1983)[L]	*P. proteus*	ND	ND	*A. rubrum*	CLL	ND	0	15	ND	0.0059
	P. proteus	ND	ND	*C. florida*	CLL	ND	0	15	ND	0.0115
	P. proteus	ND	ND	*Q. alba*	CLL	ND	0	15	ND	0.0023
	P. proteus	ND	ND	*R. pseudoacacia*	CLL	ND	0	15	ND	ND
	P. proteus	ND	ND	*A. rubrum*	CLL	ND	28	15	ND	0.0096
	P. proteus	ND	ND	*C. florida*	CLL	ND	28	15	ND	0.0322
	P. proteus	ND	ND	*Q. alba*	CLL	ND	28	15	ND	0.0071
	P. proteus	ND	CLL	*R. pseudoacacia*	CLL	ND	28	15	ND	0.0169
	P. proteus	ND	CLL	*A. rubrum*	CLL	ND	56	15	ND	0.0215
	P. proteus	ND	CLL	*C. florida*	CLL	ND	56	15	ND	0.0253
	P. proteus	ND	CLL	*Q. alba*	CLL	ND	56	15	ND	0.0103
	P. proteus	ND	CLL	*R. pseudoacacia*	CLL	ND	56	15	ND	0.017
Grafius and Anderson (1979)[L]	*L. quercina*	V	2	*A. rubra*	CLL	ND	14–21	5	0.208	0.104
	L. quercina	V	2	*A. rubra*	CLL	ND	14–21	10	0.216	0.108
	L. quercina	V	2	*A. rubra*	CLL	ND	14–21	15	0.216	0.108
Iversen (1979)[L]	*S. personatum*	II–VII	1	*F. sylvatica*	CLL	1.26 ± 0.11	40	3	0.0132	0.0132
	S. personatum	II–VII	10	*F. sylvatica*	CLL	1.26 ± 0.11	40	3	0.067	0.0067
	S. personatum	II–VII	1	*F. sylvatica*	CLL	1.26 ± 0.11	40	8	0.0336	0.0336
	S. personatum	II–VII	10	*F. sylvatica*	CLL	1.26 ± 0.11	40	8	0.166	0.0166
	S. personatum	II–VII	1	*F. sylvatica*	CLL	1.26 ± 0.11	40	13	0.0467	0.0466
	S. personatum	II–VII	10	*F. sylvatica*	CLL	1.26 ± 0.11	40	13	0.233	0.0233
	S. personatum	II–VII	1	*F. sylvatica*	CLL	1.26 ± 0.11	40	18	0.0545	0.0549
	S. personatum	II–VII	10	*F. sylvatica*	CLL	1.26 ± 0.11	40	18	0.272	0.0272
Jacobsen and Sand-Jensen (1994)[L]	*A. nervosa*	V	18.15 ± 0.81	*A. glutinosa*	UGL	3.68 ± 0.15	2	15	0.479[C]	0.0264[C]
	A. nervosa	V	18.15 ± 0.81	*A. glutinosa*	UGL	3.68 ± 0.15	2	15	0.018[N]	0.000992[N]
	A. nervosa	V	18.15 ± 0.81	*F. sylvatica*	UGL	3.48 ± 0.30	2	15	0.377[C]	0.0208[C]
	A. nervosa	V	18.15 ± 0.81	*F. sylvatica*	UGL	3.48 ± 0.30	2	15	0.012[N]	0.000661[N]
	A. nervosa	V	18.15 ± 0.81	*P. perfoliatus*	UGM	3.68 ± 0.11	2	15	0.471[C]	0.0260[C]
	A. nervosa	V	18.15 ± 0.81	*P. perfoliatus*	UGM	3.68 ± 0.11	2	15	0.022[N]	0.00121[N]
Otto (1974)[L]	*P. cingulatus*	III	2.9	*A. glutinosa*	CLL	ND	4	1.8	0.0893	0.0308
	P. cingulatus	III	4.2	*A. glutinosa*	CLL	ND	4	1.8	0.0956	0.0228
	P. cingulatus	V	5	*A. glutinosa*	CLL	ND	4	1.8	0.0888	0.0178
	P. cingulatus	V	5.5	*A. glutinosa*	CLL	ND	4	1.8	0.0883	0.016
	P. cingulatus	I–II	0.15	*A. glutinosa*	CLL	ND	4	5	0.0335	0.224
	P. cingulatus	III	1.1	*A. glutinosa*	CLL	ND	4	5	0.109	0.0987
	P. cingulatus	V	8.2	*A. glutinosa*	CLL	ND	4	5	0.274	0.0334

Table 2. continued

Citation	Species	Instar	Size class (mg)	Food source	LT	LNC (%N)	CT (d)	WT (°C)	ARI	RAR
	P. cingulatus	V	16.3	*A. glutinosa*	CLL	ND	4	10	0.728	0.0446
	P. cingulatus	V	25.1	*A. glutinosa*	CLL	ND	4	15	0.821	0.0327
	P. cingulatus	V	29	*A. glutinosa*	CLL	ND	4	15	0.677	0.0233
Perry and others (1987)[L]	*P. proteus*	ND	5–25	*A. rubrum*	CLL	ND	28 + 7*	5	0.227	0.0227
	P. proteus	ND	40–70	*A. rubrum*	CLL	ND	28 + 7*	5	0.744	0.0149
	P. proteus	ND	90–130	*A. rubrum*	CLL	ND	28 + 7*	5	1.127	0.0113
	P. proteus	ND	180–250	*A. rubrum*	CLL	ND	28 + 7*	5	2.184	0.0149
	P. proteus	ND	5–25	*A. rubrum*	CLL	ND	28 + 7*	10	0.258	0.0258
	P. proteus	ND	40–70	*A. rubrum*	CLL	ND	28 + 7*	10	0.847	0.0169
	P. proteus	ND	90–130	*A. rubrum*	CLL	ND	28 + 7*	10	1.43	0.0143
	P. proteus	ND	180–250	*A. rubrum*	CLL	ND	28 + 7*	10	2.717	0.0109
	P. proteus	ND	5–25	*A. rubrum*	CLL	ND	28 + 7*	20	0.177	0.0177
	P. proteus	ND	40–70	*A. rubrum*	CLL	ND	28 + 7*	20	2.00928	0.0402
	P. proteus	ND	90–130	*A. rubrum*	CLL	ND	28 + 7*	20	2.976	0.0298
	P. proteus	ND	180–250	*A. rubrum*	CLL	ND	28 + 7*	20	5.431	0.0136
This study[F]	*H. designatus*	IV	1.9 ± 0.43	*P. fremontii*	ULL	2.26 ± 0.22	0	8.3 ± 0.75	0.00677[C]	0.00287[C]
	H. designatus	IV	1.9 ± 0.43	*P. fremontii*	ULL	2.26 ± 0.22	0	8.3 ± 0.75	0.000417[N]	0.000214[N]
	H. designatus	IV	1.9 ± 0.43	*P. angustifolia*	ULL	2.34 ± 0.19	0	8.3 ± 0.75	0.0150[C]	0.00586[C]
	H. designatus	IV	1.9 ± 0.43	*P. angustifolia*	ULL	2.34 ± 0.19	0	8.3 ± 0.75	0.00194[N]	0.000740[N]
	Micrasema sp.	IV	0.52 ± 0.097	*P. fremontii*	ULL	2.26 ± 0.22	0	8.3 ± 0.75	0.000117[C]	0.000253[C]
	Micrasema sp.	IV	0.52 ± 0.097	*P. fremontii*	ULL	2.26 ± 0.22	0	8.3 ± 0.75	8.56E−06[N]	0.0000189[N]
	Micrasema sp.	IV	0.52 ± 0.097	*P. angustifolia*	ULL	2.34 ± 0.19	0	8.3 ± 0.75	0.000262[C]	0.000647[C]
	Micrasema sp.	IV	0.52 ± 0.097	*P. angustifolia*	ULL	2.34 ± 0.19	0	8.3 ± 0.75	0.0000182[N]	0.0000475[N]
	Antocha sp.		0.20 ± 0.048	*P. fremontii*	ULL	2.26 ± 0.22	0	8.3 ± 0.75	0.000153[C]	0.000692[C]
	Antocha sp.		0.20 ± 0.048	*P. fremontii*	ULL	2.26 ± 0.22	0	8.3 ± 0.75	8.51E−06[N]	0.0000418[N]
	Antocha sp.		0.20 ± 0.048	*P. angustifolia*	ULL	2.34 ± 0.19	0	8.3 ± 0.75	0.0000991[C]	0.000323[C]
	Antocha sp.		0.20 ± 0.048	*P. angustifolia*	ULL	2.34 ± 0.19	0	8.3 ± 0.75	0.0000132[N]	0.0000471[N]
	Entire community			*P. fremontii*	ULL	2.26 ± 0.22	0	8.3 ± 0.75	0.000130[C]	0.000739[C]
	Entire community			*P. fremontii*	ULL	2.26 ± 0.22	0	8.3 ± 0.75	9.29E−06[N]	0.0000536[N]
	Entire community			*P. angustifolia*	ULL	2.34 ± 0.19	0	8.3 ± 0.75	0.000201[C]	0.00158[C]
	Entire community			*P. angustifolia*	ULL	2.34 ± 0.19	0	8.3 ± 0.75	0.0000259[N]	0.000201[N]

Asterisks (*) designate a case where leaves were conditioned for 4 weeks, frozen, and then soaked in aerated stream water for 1 week before being used as food. Citations followed by [L] and [F] designate estimates made in laboratory and field studies, respectively. Values followed by [C] and [N] designate element assimilation rates for carbon (mg assimilated C) and nitrogen (mg assimilated N) (insect^{-1} d^{-1} (ARI) and mg insect^{-1} d^{-1} (RAR)), respectively.

willow (*Salix exigua* Nutt.), and Goodding's willow (*S. gooddingii* Ball) (LeRoy and Marks 2006). Oak Creek has an average annual flow of 368 l s^{-1}, and the watershed contains Paleozoic sandstones and Tertiary igneous formations, giving it a relatively high alkalinity (LeRoy and Marks 2006). Temperature, pH, total dissolved solids, specific conductivity, and salinity were measured along transects at the top and bottom of the reach during each harvest of the study using a Hydrolab minisonde (Hydrolab-Hach Corporation, Loveland, Colorado, USA) (n = 10 measurements per transect). Average values across the 45-day study period were as follows (means ± standard errors): temperature (8.3 ± 0.75°C), pH (8.2 ± 0.16), dissolved oxygen (100 ± 0.76%), salinity (0.12 ± 0.005 ppt), total dissolved solids (0.160 ± 0.006 g l^{-1}), and specific conductivity (250 ± 9.2 µS cm^{-1}). Unlabeled animal tissue for each major insect taxon, representing each of our functional feeding groups, was collected before the experiment from insects at large in the stream, from above and below the study reach, to get their respective natural abundance values.

We incubated leaf packs containing labeled *P. fremontii* and labeled *P. angustifolia* cottonwood litter, paired on rebar, and distributed along a single, approximately 100-m riffle-run reach of Oak Creek. Leaf litter (2 g) was placed in 20.32 cm^2 Vexar mesh leaf packs with 4 × 10 mm mesh, allowing for insect colonization but excluding fish. Twenty replicates of each litter type were harvested on day 17 and processed for isotope analysis. We chose this time period because it allowed sufficient time for insects to colonize packs. We also harvested leaf packs (n = 20 per leaf type) on days 31 and 45 to measure decomposition rates, insect composition, and residual isotopic concentrations of litter.

Sample Processing and Decomposition

Remaining litter and aquatic insects from leaf packs were collected for each harvest. Leaf packs were processed within 48 h of harvesting. Sediment, leaves, and insects were rinsed multiple times with DI water using 250-µm sieves. Aquatic insects and remaining litter from each pack were sorted and identified before being dried at 60°C for 96 h for isotope analysis preparation. Insects were identified to the lowest taxonomic level possible (usually genus) and insects that could not be identified were morphotyped. A reference collection was made from the insects collected at large in the stream before the start of the project, which is archived at the NAU Biodiversity Center. The diets of each taxonomic group were assigned based on functional feeding group designations from published keys (e.g., Merritt and Cummins 1996). Counts of insects were recorded, and the total dry biomass of each taxonomic group was measured using a microbalance (Mettler-Toledo XP6). Dried litter was weighed and ground in a Wiley Mill to 425 µm. Subsamples were combusted at 550°C in a muffle furnace (Barnstead International, Dubuque, Iowa, USA) for 1 h to determine ash-free dry mass (AFDM) using the method of Benfield (2006).

Decomposition was estimated using exponential decay:

$$L_t = L_i e^{-kt}, \quad (1)$$

where L_t is the AFDM of remaining litter at time t, L_i is the AFDM of initial litter mass from handling packs (Benfield 2006), and k is the instantaneous decomposition rate constant. Decomposition rate constants (k) were calculated as the slope of the natural log-transformed AFDM remaining line (Benfield 2006).

Stable Isotope Analysis

Stable isotopes of C and N were used to examine nutrient flux from leaf litter to associated aquatic insects. Because of the mass requirements for stable isotope analysis of animal tissue, we were restricted to taxonomic groups that had at least approximately 0.6 mg insect tissue. The reduced data set that was used for isotope analysis represented 98% of the total taxa biomass, 98% of the total taxa abundance, and 75% of the total species of the full dataset. For isotopic analysis of litter and insects, samples were ground in a mortar and pestle for homogenization, weighed (4–6 mg for leaves, 0.6–1.2 mg for insect tissue) on a microbalance (Mettler-Toledo XP6), encapsulated in 4 × 6-mm tin cups (Costech Analytical Technologies Inc., Valencia, California, USA) and delivered to the Colorado Plateau Stable Isotope Laboratory (CPSIL) of Northern Arizona University. Litter and insect ^{13}C, ^{15}N, C, and N contents were measured using a Carlo Erba NC 2100 Elemental Analyzer (CE Instruments, Milan, Italy) with a Thermo-Finnigan Delta Plus XL (Thermo-Electron Corp., Bremen, Germany) isotope ratio mass spectrometer at CPSIL (http://www.mpcer.nau.edu/isotopelab/). Litter and insect ^{15}N and ^{13}C isotope compositions were expressed in standard delta notation ($\delta^{13}C$, $\delta^{15}N$) in parts per thousand (‰) relative to Vienna PeeDee Belemnite for C and air for N, as follows:

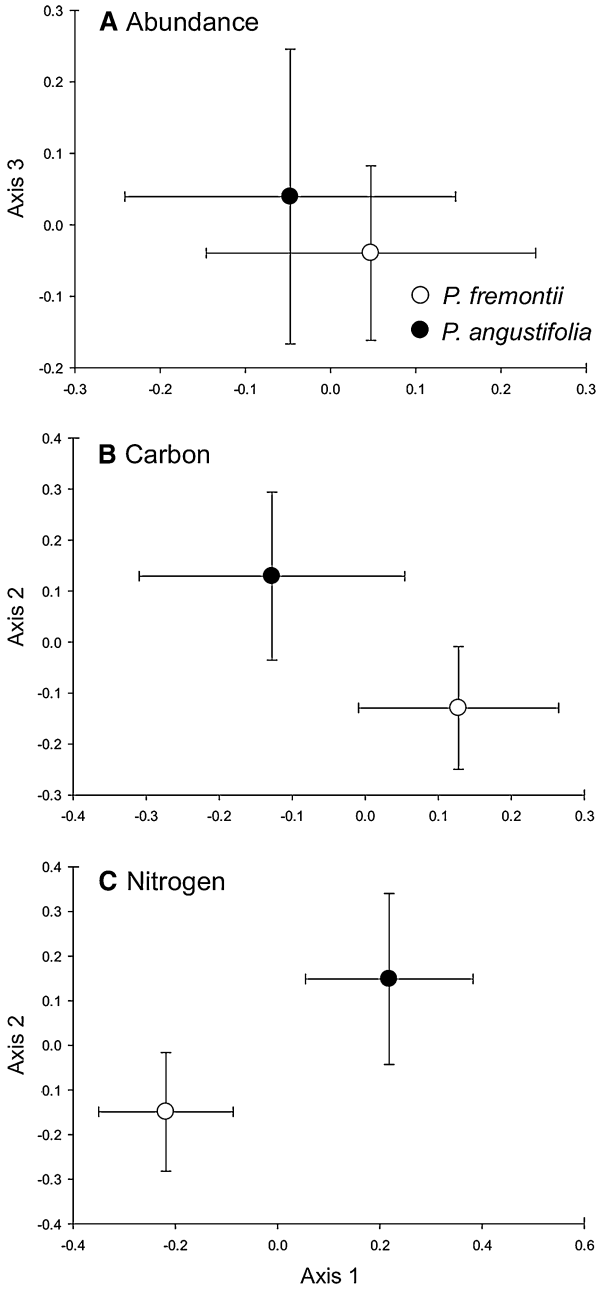

Figure 1. Non-metric multidimensional scaling (NMDS) ordinations of aquatic insect communities associated with *Populus fremontii* (*white circles*) and *P. angustifolia* (*black circles*) leaf litter. Community ordinations are based on relative taxa abundances ($A = -0.0384$, $P = 0.886$) (**A**), carbon flux values ($A = 0.0377$, $P = 0.0617$) (**B**), and nitrogen flux values ($A = 0.0487$, $P = 0.0352$) (**C**). Error bars depict standard errors of mean NMDS scores for a given leaf type.

$$\delta = 1,000 \times \left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) - 1‰, \qquad (2)$$

where R is the molar ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The external precision (standard error) on repeated

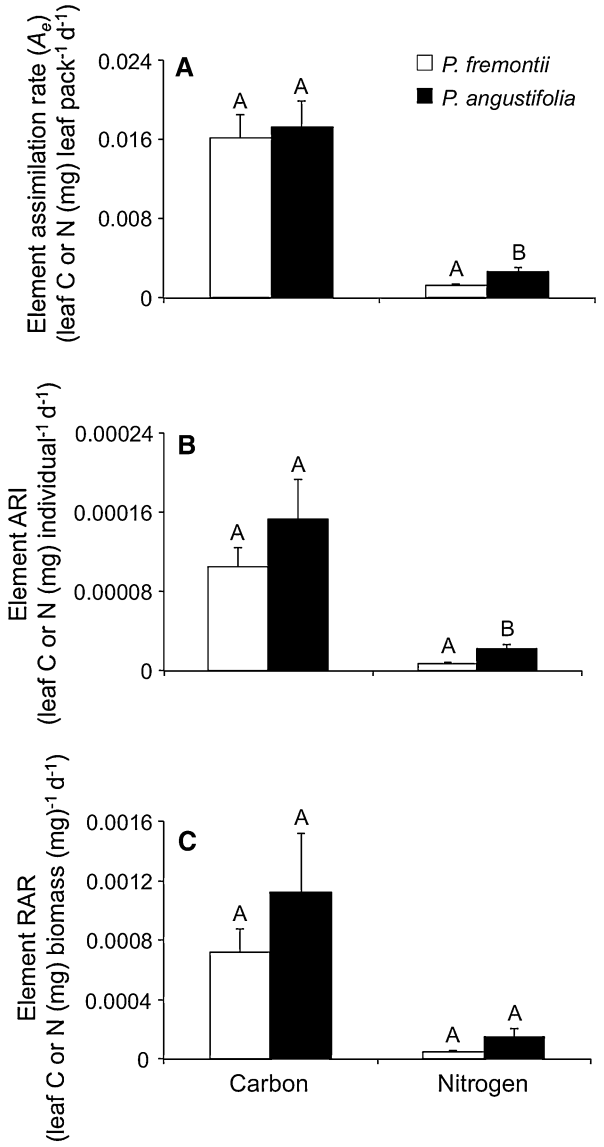

Figure 2. Community-level (at the scale of the leaf pack) element assimilation rates (A_e) (leaf C or N (mg) leaf pack^{-1} d^{-1}) of carbon and nitrogen from labeled cottonwood leaves by all taxa (**A**), and standardized by taxa abundance (element ARI) (**B**), and taxa biomass (mg) (element RAR) (**C**). Differing letters above bars depict statistical differences between *Populus fremontii* (*white*) and *P. angustifolia* (*black*) treatments using Student's *t* tests on $\log_{10}$-transformed data ($\alpha = 0.05$). Error bars depict +1 standard error.

measurements of a working laboratory standard (National Institute of Standards and Technology, USA; NIST 1547 peach leaves) was ±0.012‰ for $\delta^{13}C$, ±0.054‰ for $\delta^{15}N$, ±0.031% for %C, and ±0.006% for %N. Because errors arise using δ notation for mixing models, especially when δ values differ greatly from the natural abundance

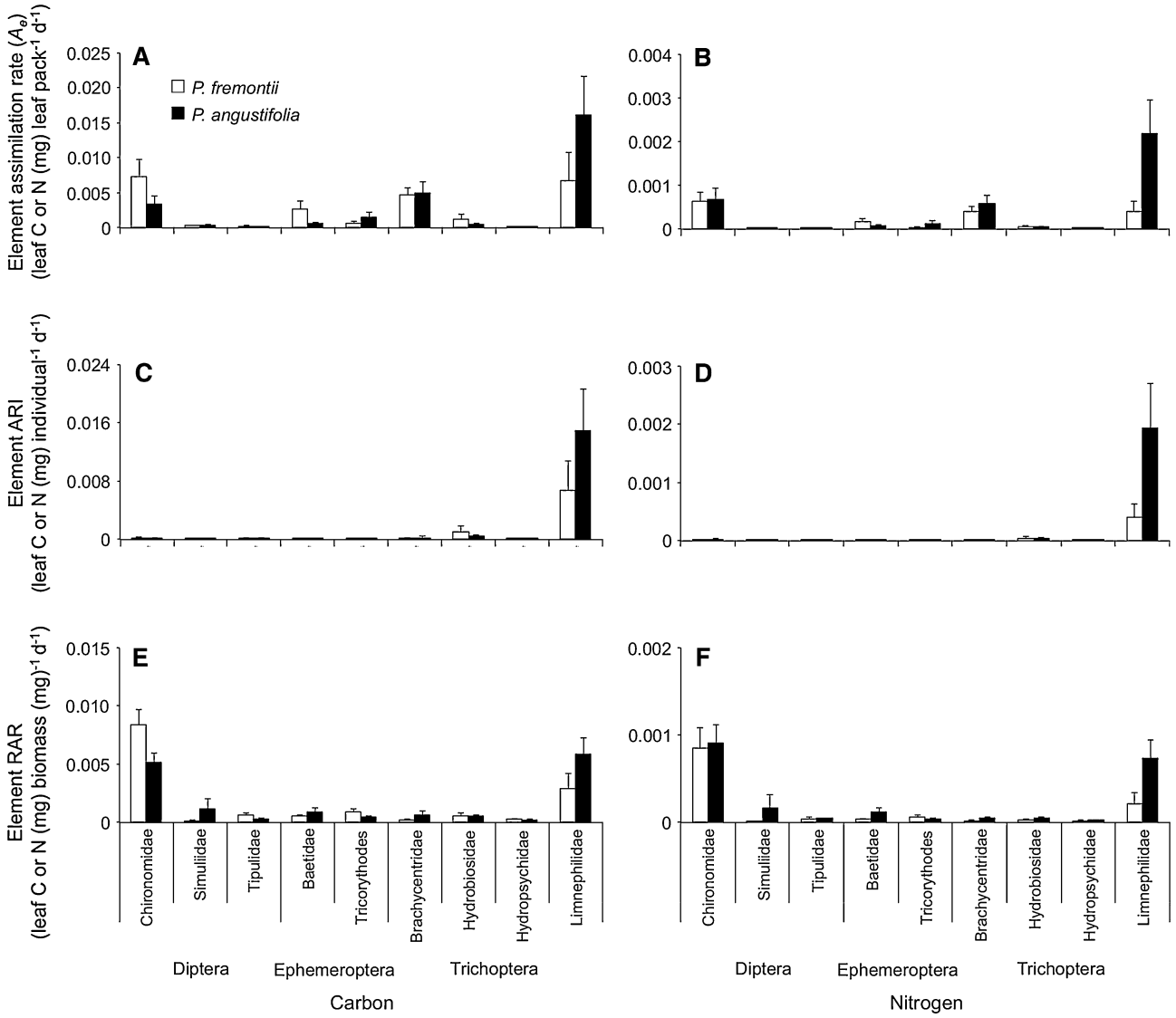

Figure 3. Element assimilation rates (A_e) (leaf C or N (mg) leaf pack^{-1} d^{-1}) of carbon (**A**) and nitrogen (**B**) from labeled *Populus fremontii* (*white bars*) and *P. angustifolia* (*black bars*) leaves by aquatic insect taxa. Overall patterns of A_e did not change when standardized by insect abundance (element ARI) for carbon (**C**) and nitrogen (**D**) or insect biomass (mg) (element RAR) for carbon (**E**) and nitrogen (**F**). Values are reported with +1 standard error.

range (Fry 2008), we used at.% values for all mixing models:

$$\text{at.}\% = \left(\frac{R_{\text{sample}}}{1 + R_{\text{sample}}}\right) \times 100\%. \quad (3)$$

We used a mass balance approach to calculate assimilation from labeled leaf litter by insects:

$$\text{Element assimilation rate } (A_e) = \frac{\left(\frac{(\text{at.}\%X_{al} - \text{at.}\%X_{ai})}{(\text{at.}\%X_{ll} - \text{at.}\%X_{ai})}\right) \times \left(M_{al} \times \left(\frac{\%X_{al}}{100}\right)\right)}{T \times M_{ll}}, \quad (4)$$

where X_{ai} is unlabeled (natural abundance) animal tissue, X_{al} is labeled animal tissue, and X_{ll} is labeled litter for a given element (for example, C or N), M_{al} is the mass of the labeled animal (μg), M_{ll} is the initial mass of labeled litter (g), $\%X_{al}$ is the percent of element X in the tissue of a labeled animal, and T is time (days). In addition to element assimilation rates (A_e) for C and N, we also calculated element assimilation rate per individual (ARI: A_e standardized to taxa abundance),

$$\mathrm{ARI} = \frac{\left(\frac{(\mathrm{at.\%}X_{\mathrm{al}} - \mathrm{at.\%}X_{\mathrm{ai}})}{(\mathrm{at.\%}X_{\mathrm{ll}} - \mathrm{at.\%}X_{\mathrm{ai}})}\right) \times \left(M_{\mathrm{al}} \times \left(\frac{\%X_{\mathrm{al}}}{100}\right)\right)}{T \times M_{\mathrm{ll}} \times Ab_t}, \quad (5)$$

where Ab_t is the abundance of taxa (t), and element relative assimilation rate (RAR: A_e standardized to taxa biomass),

$$\mathrm{RAR} = \frac{\left(\frac{(\mathrm{at.\%}X_{\mathrm{al}} - \mathrm{at.\%}X_{\mathrm{ai}})}{(\mathrm{at.\%}X_{\mathrm{ll}} - \mathrm{at.\%}X_{\mathrm{ai}})}\right) \times \left(M_{\mathrm{al}} \times \left(\frac{\%X_{\mathrm{al}}}{100}\right)\right)}{T \times M_{\mathrm{ll}} \times Bm_t}, \quad (6)$$

where Bm_t is the biomass of taxa (t), for C and N.

Element assimilation rate (equation 4) overestimates biological assimilation, defined as energy digested and assimilated into the blood stream (Chapin and others 2002), because stomach contents of insects were included in insect isotopic samples (i.e., some C and N gut contents will likely be excreted). We assessed how including gut contents overestimated A_e for a subset of samples of *Baetis* sp. (Baetidae, collector–gatherer), *Hesperophylax designatus* (Limnephilidae, shredder), and *Atopsyche* sp. (Hydrobiosidae, predator). We dissected each insect to separate insect tissue from gut contents, and we determined the mass and δ^{15}N and δ^{13}C content of the separated tissue and gut content samples. We then calculated A_e as described above (equation 4), using the mass and isotope composition of the entire sample (insect + gut contents), and compared this to A_e calculated using the mass and isotope composition of the insect tissue alone. Differences between A_e calculated from whole insect compared to non-gut tissue were small, ranging from −0.84 to 0.87% for carbon and −1.64 to 0.48% for nitrogen, and in no case were these different between *P. fremontii* and *P. angustifolia* litter for any of the insect groups (all $P > 0.31$). Thus, including the gut contents in the mass and isotope analyses had negligible impact on our estimates of assimilation.

Other studies have measured assimilation indirectly, in the laboratory, defining it as the sum of growth (or production) and respiration, with negligible losses assumed due to excretion (e.g., Otto 1974; Iversen 1979; Jacobsen and Sand-Jensen 1994; Table 2). These studies report assimilation rates per individual (ARI (mg insect^{-1} d^{-1})) because they usually involve the energetics of a single taxon (Table 2). Standardizing to the biomass of a particular taxon gives its relative assimilation rate (RAR (mg mg insect^{-1} d^{-1})), which is comparable across taxa (Table 2). Laboratory studies also often estimate assimilation efficiency (AE; Table 2), defined as the proportion of assimilation of a consumer per leaf mass ingested (Golladay and others 1983; Perry and others 1987), or the efficiency of conversion of ingested food (ECI: Lawson and others 1984; Tuchman and others 2002), defined as the proportion of biomass gained by a consumer per leaf mass ingested (Waldbauer 1968). Our method differs from traditional methods of estimating assimilation because, rather than total mass, our estimates of ARI and RAR quantify specific elements of ingested food (that is, C and N).

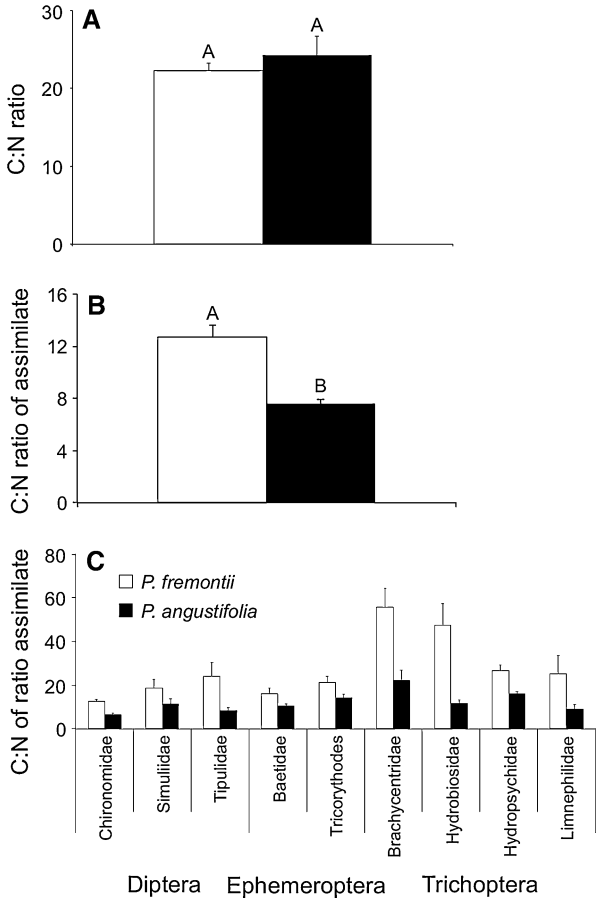

Figure 4. C:N ratios of *Populus fremontii* (*white*) and *P. angustifolia* (*black*) litter (**A**), the C:N of the assimilate provided to the aquatic insect community (at the scale of the leaf pack) (**B**), and the C:N of the assimilate provided to aquatic insect taxa (nested ANOVA: full model: $F_{19,209} = 5.54$, $P \leq 0.0001$, Leaf Species[Taxa]: $F_{10,209} = 5.94$, $P < 0.0001$) (**C**). *Differing letters above bars* depict statistical differences between treatments using Student's t tests on $\log_{10}$-transformed data ($\alpha = 0.05$). *Error bars* depict +1 standard error.

Statistical Analyses

Non-metric multi-dimensional scaling (NMDS) using Bray–Curtis distances was conducted using PC-ORD version 6 for Windows (McCune and

Mefford 2011) to examine traditional, descriptive insect communities (based on relativized abundances) and functional communities (based on carbon and nitrogen fluxes from leaves to insects). To determine whether descriptive and functional insect community composition differed between leaf type, blocked multi-response permutation procedure (MRPP) tests were conducted in PC-ORD, with leaf type as the grouping factor and rebar (for example, station in stream) as the blocking factor. NMDS and MRPP analyses were performed for both raw and relativized data for insect taxa abundances and carbon and nitrogen fluxes, as well as for both full (FCM) and reduced community matrices (RCM). Using raw data did not change the NMDS ordination patterns or statistical inferences (based on MRPP) observed from the relativized data matrices. Reduced community matrices included only the taxa that went into the community analyses for isotope flux values, which was reduced because of the mass requirement for stable isotope analysis. Therefore, using the RCM allowed for a more fair comparison of traditional communities (based on insect taxa abundances) and functional communities (based on carbon and nitrogen flux values from leaf litter to insects).

We used Student's *t* tests in JMP Pro version 10.0 (SAS Institute Inc., Cary, NC, 1989–2012) to test for taxa- and community-level (that is, leaf-pack level) A_e differences for C and N, C:N assimilate ratios (the ratio of C:N assimilated directly from labeled leaf litter, C A_e/N A_e), and taxa community parameters (abundance, biomass, species richness, and diversity). Additionally, we used nested ANOVA tests (with leaf species nested within taxa) tests to examine overall A_e differences for C, N, and C:N assimilate ratios across all taxa. A_e and C:N data were $\log_{10}$-transformed to meet assumptions of normality and equal variance (Sokal and Rohlf 1995). We did not use Bonferroni corrections across individual taxa-level comparisons because this often inflates the chance of committing type II errors (Gotelli and Ellison 2004).

ANCOVA, with a Leaf Species × Time interaction (to test for differences of slopes), was used to test decomposition rate constant (k, d^{-1}) differences between *P. fremontii* and *P. angustifolia* litter treatments. To test for normality for ANCOVA tests, Shapiro–Wilks goodness of fit tests were conducted on model residuals (Sokal and Rohlf 1995). Analysis of leaf litter decay rates (k) required natural-log transformation of AFDM data in order to meet the assumptions of normality and equal variance, and to determine exponential decay rates (k) of ln AFDM remaining (Benfield 2006).

Results

Abundance and Assimilation-Based Communities

When communities were described by the relative abundances of insect taxa, insect community composition did not differ between the two plant species, whether using the reduced community matrix (RCM) (MRPP: $A = 0.0029$, $P = 0.42$, Figure 1A) or the full community matrix (FCM) (MRPP: $A = 0.00060$, $P = 0.46$). In contrast, communities described by relativized taxon-specific rates of element assimilation differed between species for carbon (MRPP: $A = 0.032$, $P = 0.091$, Figure 1B) and nitrogen (MRPP: $A = 0.046$, $P = 0.039$, Figure 1C) fluxes.

Other traditional, descriptive community metrics based on the relative abundances of taxa were not sensitive to differences between *P. angustifolia* and *P. fremontii* litter, including abundance (RCM: $t_{18} = -1.24$, $P = 0.23$; FCM: $t_{18} = -1.19$, $P = 0.25$), biomass (RCM: $t_{18} = -0.74$, $P = 0.47$; FCM: $t_{18} = -0.66$, $P = 0.52$), species richness (RCM: $t_{18} = 0.00$, $P = 1.00$; FCM: $t_{18} = 0.35$, $P = 0.73$), species evenness (RCM: $t_{18} = 0.013$, $P = 0.99$; FCM: $t_{18} = -0.10$, $P = 0.92$), and diversity (Shannon's H: RCM: $t_{18} = -0.05$, $P = 0.96$; FCM: $t_{18} = 0.26$, $P = 0.80$; Simpson's D': RCM: $t_{18} = -0.24$, $P = 0.82$; FCM: $t_{18} = -0.12$, $P = 0.91$). However, biodiversity based on taxon-specific rates of element assimilation showed clear differences between the leaf litter types, for both carbon (Shannon's H: $t_{18} = 2.34$, $P = 0.031$; Simpson's D': $t_{18} = 2.22$, $P = 0.040$) and nitrogen (Shannon's H: $t_{18} = 2.25$, $P = 0.040$; Simpson's D': $t_{18} = 2.06$, $P = 0.054$). In other words, the community of organisms actually assimilating elements from the decomposing leaves was more diverse on *P. angustifolia* compared to *P. fremontii* litter.

Higher C and N Fluxes to Insects from *P. angustifolia*

Contrary to our hypothesis that rates of C and N assimilation would be faster from *P. fremontii* compared to *P. angustifolia* litter, nitrogen A_e was 87% higher on *P. angustifolia* than on *P. fremontii* litter ($t_{24.6} = 2.74$, $P = 0.011$), but not different for C ($t_{37.7} = 0.27$, $P = 0.79$; Figure 2A) at the community-level. Differences between leaf species were also more apparent for N when rates of element assimilation (A_e) were expressed on a per capita basis (C: $t_{28.0} = 1.12$, $P = 0.27$; N: $t_{20.9} = 2.86$, $P = 0.0093$; Figure 2B) or per unit biomass

(C: $t_{26.0} = 0.94$, $P = 0.36$; N: $t_{20.1} = 2.00$, $P = 0.060$; Figure 2C).

At the taxa level, C and N assimilation rates (A_e) were higher from *P. angustifolia* litter (for C, nested ANOVA: full model: $F_{17,105} = 7.96$, $P < 0.0001$, Leaf Species[Taxa]: $F_{9,105} = 2.23$, $P = 0.0258$, Figure 3A; for N, nested ANOVA: full model: $F_{17,105} = 8.00$, $P < 0.0001$, Leaf Species[Taxa]: $F_{9,105} = 2.56$, $P < 0.0106$, Figure 3B). These patterns were consistent when expressed per individual (ARI; C: nested ANOVA: full model: $F_{17,105} = 8.33$, $P < 0.0001$, Leaf Species[Taxa]: $F_{9,105} = 2.03$, $P < 0.0431$, Figure 3C; N: nested ANOVA: full model: $F_{17,105} = 7.00$, $P < 0.0001$, Leaf Species[Taxa]: $F_{9,105} = 4.24$, $P = 0.0001$, Figure 3D) or per unit biomass (RAR; C: nested ANOVA: full model: $F_{17,105} = 13.2$, $P < 0.0001$, Leaf Species[Taxa]: $F_{9,105} = 1.71$, $P = 0.0957$, Figure 3E; N: nested ANOVA: full model: $F_{17,105} = 11.9$, $P < 0.0001$, Leaf Species[Taxa]: $F_{9,105} = 4.91$, $P = 0.0059$, Figure 3F). These patterns occurred despite *P. fremontii* litter decomposing significantly faster than *P. angustifolia* litter (ANCOVA: full model: $F_{3,44} = 122$, $P < 0.0001$, Leaf Species × Time: $t = -3.44$, $P = 0.0013$, Table 1).

In general, differences between plant species in A_e were much larger for N than for C, as five of nine taxa had significantly higher N A_e values from *P. angustifolia* than *P. fremontii* litter, whereas no significant differences were found for C (Figure 3). In addition to Baetidae (predominantly collector–gatherers) and Hydrobiosidae (exclusively predators) taxa, all shredder taxa assimilated more N from *P. angustifolia* litter (with the exception of Tipulidae taxa, which did not significantly differ in A_e values) (Figure 3). Patterns were strongest for the dominant shredder in our system, *Hesperophylax designatus* (Limnephilidae), which assimilated 1.4 times more C (Figure 3A) and 4 times more N from *P. angustifolia* (Figure 3B) than from *P. fremontii* litter, respectively.

Mismatch of C:N of Leaves Versus C:N of Insect Assimilate

There was a mismatch between the stoichiometry of leaf litter material and the stoichiometry of element assimilation by aquatic invertebrates growing on that litter. At the community-level, the C:N ratio of the assimilate for the entire leaf pack was approximately 69% lower from *P. angustifolia* compared to *P. fremontii* ($t_{31.3} = -5.24$, $P < 0.0001$; Figure 4B). In contrast to the C:N ratios of labeled leaf assimilate found in insects (that is, the assimilate of insect bodies coming directly from labeled leaves), the C:N of whole insects (that is, the total C:N of insect bodies) did not differ based on litter type (nested ANOVA: full model: $F_{24,224} = 5.31$, $P < 0.0001$, Leaf Species[Taxa]: $F_{12,224} = 0.62$, $P = 0.82$; Taxa: $F_{12,224} = 9.40$, $P < 0.0001$).

This pattern was also apparent at the taxa level. Assimilate C:N ratios of insects were lower on *P. angustifolia* compared to *P. fremontii* across all taxa (nested ANOVA: full model: $F_{19,209} = 5.54$, $P < 0.0001$, Leaf Species[Taxa]: $F_{10,209} = 5.94$, $P < 0.0001$; Figure 4C) despite similar initial C:N ratios of the litter types ($t_{101.9} = 0.80$, $P = 0.43$; Figure 4A; Table 1). Mean C:N ratios of the litter-derived assimilate to insect taxa were lower for *P. angustifolia* litter, with 6 of 9 taxa demonstrating significant differences between *P. fremontii* and *P. angustifolia* litter (Figure 4C). The magnitude of differences ranged from ~50% lower for collector–gatherer and collector–filterer taxa to around 300–350% lower for some shredders on *P. angustifolia* compared to *P. fremontii* litter.

Discussion

Utility of Isotopic Tracers for Resolving the Functioning of Insect Communities

This study shows that labeled leaf litter provides a more sensitive measurement than insect counts for assessing the role litter type plays in stream food webs, circumventing two key—but rarely met—assumptions of classical biodiversity measures: relative abundances establish the importance of species (that is, all species are equal) and all individuals are equal (Mouchet and others 2010). Similar to other studies of streams in the Southwest (LeRoy and Marks 2006; LeRoy and others 2006, 2007), in our study insect colonization (that is, abundance, species richness, evenness, and diversity) and community composition (based on relative abundance values) indicated that insects did not discriminate between litter types. However, our isotope labeling technique revealed significant differences between plant species in their C and N transfer from litter to aquatic insects and their functional communities (based on element flux values).

Plant Species Alter C and N Flux to Aquatic Insects

Our finding that *P. angustifolia* litter provided more C and N to aquatic insects than *P. fremontii* litter was surprising, because *P. angustifolia* decomposes more slowly than *P. fremontii*. Two mechanisms

may account for this result. First, leaf chemistry may have mediated differences in bacterial and fungal colonization. Second, compounds in fast-decomposing litter may have leached out or have been decomposed by microbes before they were available to aquatic insects, diminishing the nutritional quality of fast-decomposing leaf litter to insects.

The first mechanism involves the relative roles of bacteria and fungi in modifying and subsidizing litter quality. Fungi, which can provide more nutrition to aquatic shredders compared to bacteria (Findlay and others 1986), are expected to have a greater role in the decomposition of slow-decomposing leaves (Singh 1982; Chamier 1985; Abdullah and Taj-Aldenn 1989; Au and others 1991) and wood (Zare-Maivan and Shearer 1988; Yuen and others 1998; Abdel-Raheem and Shearer 2002; Bucher and others 2004) in streams because they have enzymes that break down lignin, cellulose, and hemi-cellulose. The morphology of fungal wood decay has been observed on freshwater-exposed wood (Zare-Maivan and Shearer 1988; Yuen and others 1999), and because bacterial wood decay is superficial (Holt and Jones 1983; Eslyn and Moore 1984; Singh and Butcher 1991) relative to fungal decay, the role of fungi is thought to be more important. Fungi can penetrate recalcitrant substrates by creating extensive hyphal networks, which bypass recalcitrant outer leaf litter layers, and forage for nutrients, excreting a cocktail of extracellular enzymes that degrade organic matter (Leake and Read 1997; de Boer and others 2005; van der Heijden and others 2008). Fungi may also alleviate nutrient limitations of litter decomposition through reallocation of nutrients (Hendrix and others 1986; Holland and Coleman 1987) and by acting as vectors for bacterial transport, expediting bacterial colonization of new substrates (Kohlmeier and others 2005). Collector–gatherers and shredders, then, can take advantage of fungi by direct consumption or by accessing reallocated nutrients from slow-decomposing leaves. Consequently, leaves of *P. angustifolia*, by decomposing more slowly, may have provided a more stable, high-quality resource through time. Data from related studies support this mechanism, where higher fungal colonization and greater fungal:bacteria ratios (Wymore and others 2013) and higher fungal diversity (Marks and others 2009) were observed on *P. angustifolia* compared to *P. fremontii* litter.

A second mechanism explaining the finding that insects assimilated C and N at a faster rate from *P. angustifolia* compared to *P. fremontii* litter is that fast-decomposing litter may lose soluble compounds before aquatic insects can colonize and utilize it, altering its effective ''quality'' to those insects compared to what is inferred from intact litter and decomposition studies. Consistent with this, *P. fremontii* litter leached significantly more dissolved organic carbon than *P. angustifolia* litter (Wymore and others 2014). Dissolved organic compounds, which generally have very long transport distances downstream (Ensign and Doyle 2006), are unavailable to most microbes and insects associated with leaf litter. Leaching could have been accelerated in our study because of rapid decomposition. We found decomposition rates (k, d^{-1}) between 3.4 and 5.6 times higher than other studies that occurred at the same time of year and used the same litter species (LeRoy and others 2006, 2007; Table 1). Our litter decomposition rates were higher than those from previous studies likely because our litter was grown in the greenhouse, resulting in our leaf litter having nitrogen concentrations more comparable to those of conditioned or fresh litter used in laboratory studies (Table 2) than concentrations of wild senescent leaf litter (Table 1). Although lignin and tannins may retard nutrient cycling in slow-decomposing leaf litter, our results suggest the slower release rates of energy and nutrients bound in complex chemicals may increase their transfer efficiency and absolute rate of transfer to insects.

Isotopic Assimilate C:N Does Not Correspond to Litter C:N

Despite similar initial C:N ratios, insects assimilated more nitrogen (relative to carbon) from *P. angustifolia* compared to *P. fremontii* litter, suggesting higher nutritional quality of *P. angustifolia*. One possible mechanism explaining this counterintuitive finding is that *P. fremontii* litter loses relatively more N during leaching. As decomposition progresses, *P. fremontii* litter remains as a leaf skeleton, including only the vasculature of the petiole and leaf blade. As this leaf skeleton is primarily recalcitrant carbon, it is so depleted of nitrogen that its nutritional value is minimal. In contrast, *P. angustifolia* litter releases nutrients more slowly. This structural, slow-release mechanism, while retarding the absolute rate of mass loss, may preserve a C:N stoichiometry that more closely matches nutrient requirements of aquatic insects.

In our experiment, insects acquired C and N in ratios differing from those of the two litter sources and in ratios differing between the two litter sources, while maintaining constant C:N ratios in their body tissue. Aquatic insects can adjust their

consumption rates when food quality is low (House 1965; Schindler 1971) or regulate internal elemental composition by varying the excretion of nitrogen based on the nutrient content of their food (Balseiro and Albariño 2006). Detritivores contain relatively more N than occurs in terrestrial plant material, which puts stoichiometric constraints on their growth and reproduction (Elser and others 2000; Cross and others 2003; Balseiro and Albariño 2006). In fact, the C:N imbalance between shredders and their terrestrial food is likely the most disproportionate among all organism-resource components in food webs (Cross and others 2003, Evans-White and others 2005). Despite this large mismatch, aquatic insects are thought to have stable atomic C:N ratios (Frost and others 2003; Evans-White and others 2005; Balseiro and Albariño 2006). Our data show that insects maintained similar C:N ratios in their body tissue despite different assimilation rates from the two litter types. If insect internal N concentrations are tightly regulated, they have to adjust assimilation ratios or excretion ratios as litter ratios change. Additionally, as this was a field study, insects had access to food particles drifting into litter bags, potentially complimenting nutrient acquisition from leaves.

Element Assimilation Rates (A_e) Compared to Laboratory Assimilation Rates

Rates of assimilation measured in our study were lower than those typically measured in the laboratory (Table 2). One factor that may have contributed to the low assimilation rates found in this study is that immigration and emigration rates were not considered. Insects moving in and out of litter packs could have diluted the $\delta^{13}C$ and $\delta^{15}N$ signal detected from the consumers on the packs. The mesh size of packs in this study (4 × 10 mm) was sufficient to allow insects to colonize packs, but organisms that grew larger than the mesh size once inside the packs would have been retained while small organisms could have emigrated from the leaf packs. If movement in and out of packs by small organisms was significant, then the apparent assimilation of labeled C and N would have been lower in small organisms due to the continuous colonization of new unlabeled individuals. Additionally, different rates of tissue turnover among taxa could have influenced our comparisons of assimilation rates between taxa. Because we estimated assimilation based on one time point measurement of mass and isotope composition, we might have underestimated assimilation rates in organisms that turn over rapidly. The central concern of this paper, however, was to compare assemblages of insects assimilating carbon and nitrogen between two different litter types, and differential turnover among taxa is less a concern for this comparison, particularly given the strong overlap in community composition among litter types.

Despite the inherent limitations of field litter pack studies, we submit that our assimilation estimates are more realistic than those typically estimated in the laboratory for two reasons. First, we measured assimilation rates in the field, using unprocessed, whole leaves, whereas past studies measured assimilation in the laboratory, using preconditioned, often green leaves (Jacobsen and Sand-Jensen 1994; Friberg and Jacobsen 1994, 1999) or leaf disks (for example, Iversen 1979; Perry and others 1987), which omit important leaf components, such as recalcitrant vasculature and soluble compounds (Table 2). Our approach was more realistic because it simulated leaf litter that falls into the stream, followed by leaching before insect colonization. Second, our assimilation rates incorporated insect colonization times and alternative food sources, both factors that add realistic variation but are not considered in laboratory studies. By using whole, senescent leaf litter that was conditioned by microbes and insects simultaneously and by allowing for other food sources, our study provides realistic estimates of assimilation in a natural stream setting. Low assimilation rates for C and N by shredders in natural conditions underscores the importance of shredders as inefficient feeders (Oertli 1993) that aid in making terrestrial litter available to the rest of the aquatic food web (Cummins and others 1989).

Implications for Food Webs and Nutrient Cycling

Despite the difficulty of disentangling form from function in biodiversity studies (Naeem and Wright 2003), ecologists have continued to make functional inferences based on taxa abundances, likely because of the difficulty of measuring functional traits. Our finding that the communities found on leaf litter do not entirely reflect the functions insects are performing has implications for how we view important ecological processes, such as decomposition and nutrient transfer. Many of the studies that found plant species differ in decomposition rate but not in the abundance, richness, diversity (for example, LeRoy and Marks 2006;

LeRoy and others 2006), and composition (for example, LeRoy and others 2007; Li and others 2009; Dudgeon and Gao 2011) of aquatic insects could have actually had associated communities that were performing different functions, or functions at different rates, masking relationships with decomposition and nutrient transfer.

Quantifying the functional relationships that structure food webs (Paine 1980; Power 1995) has long been thought to be more incisive than describing food webs as networks of putative interactions based on the co-occurrences of potentially non-interacting taxa. Yet, ever since accessible texts (for example, Gauch 1982; McCune and others 2002; Clarke and Gorley 2006) and software (for example, PCOrd, PRIMER) made ordination techniques available to the average practitioner, it has been common, even vogue, to describe ecological communities using multidimensional data reduction techniques where the underlying data are simply relative abundances of co-occurring taxa. In such cases, findings of ''no effect'' may obscure underlying functional relationships that influence material and energy flow. We have shown how combining measures of taxa abundances with isotope tracers can quantify element transfer from decomposing leaves to aquatic insects, and we submit that this approach will improve our understanding of the relationships between food web structure and element cycling in aquatic ecosystems.

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